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The effect of caffeic acid phenethyl ester on bacterial translocation and intestinal damage after intestinal obstruction

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Aim: Intestinal obstruction (IO) induces bacterial translocation due to failure of the intestinal barrier function. Following bacterial overgrowth, its degradation products play a decisive role in the development of systemic septic complications. The aim of this study was to evaluate the effects of caffeic acid phenethyl ester (CAPE) on bacterial translocation and intestinal damage in an IO model in rats.

Materials and methods: Complete IO was created in the distal ileum of rats by a single 4-0 silk suture. A total of 21 Wistar albino rats were randomized into 3 groups: Group 1, Sham (n = 7); Group 2, IO (n = 7); Group 3, IO + CAPE (n = 7). Group 3 received a 10 μmol kg-1 dose of CAPE intraperitoneally. This treatment was continued for 3 days (2 days before surgery and 1 day after surgery). Samples of mesenteric lymph nodes (MLN), liver, and segmental ilea were obtained 24 h after the mechanical bowel obstruction, both for biochemical analysis and microbiological examination.

Results: The most common bacteria cultured from the liver and MLN of these animals were *Escherichia coli, Proteus mirabilis,* and *Enterococcus* spp. In the CAPE-treated rats, the malondialdehyde (MDA) and adrenomedullin levels were significantly lower than in the IO group ($P < 0.001$). The reduced glutathione (GSH) and catalase (CAT) levels of the ileum were found to be significantly higher in the CAPE-treated rats than those in the IO group ($P < 0.001$).

Conclusion: These results have shown that CAPE may have protective effects against bacterial translocation and intestinal oxidative damage in mechanical IO. More experimental studies are needed to explain the exact mechanism of this beneficial effect.

Key words: Intestinal obstruction, bacterial translocation, caffeic acid phenethyl ester, oxidative stress, adrenomedullin

Cafeic acid phenethyl esterin intestinal obstruksiyon sonrası bakteriyel translokasyon ve intestinal hasar üzerindeki etkisi

Amaç: Obstruksiyon intestinal koruyucu bariyeri bozarak bakteriyel tranlokasyona sebep olur. Bu bakterilerin çoğalması ve yıkım ürünleri sonucu septik komplikasyonlar gelişir. Bu çalışmanın amacı cafeic acid phenethyl esterin (CAPE) ratlarda oluşturulan intestinal obstruksiyon modelinde bakteriyel translokasyon ve intestinal hasar üzerindeki etkisini araştırmaktır.

Yöntem ve gereç: Ratlarda intestinal obstruksiyon (İO) distal ileumun 4/0 ipek sütürle bağlanması ile sağlandı. 21 Wistar albino rat üç gruba ayrıldı. Grup 1: Sham (n = 7), grup 2: İO (n = 7), grup 3: İO + CAPE (n = 7). Üçüncü gruba günde 10 μmol kg-1 dozunda CAPE intestinal obstruksiyon oluşturulmadan iki gün önce başlanarak üç gün intraperitonel verildi. İntestinal obstruksiyondan 24 saat sonra ratlar sakrifiye edildi. Ratların mezenterik lenf nodu, karaciğer ve ilumundan örnek alınarak biyokimyasal analiz ve mikrobiyolojik inceleme yapıldı.

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Bulgular: Mezenterik lenf nodu ve karaciğerden alınan örneklerin bakteriyel kültür ortamına ekimde en sık üreyen mikroorganizmalar *E. coli, Proteus mirabilis* ve *Enterococcus* spp. idi. Grup 3'te malondialdehyde (MDA) ve adrenomedullin seviyeleri grup 2' ye göre istatistiksel olarak anlamlı derecede düşük bulundu (P < 0,001). İleumdan alınan örnekte reduced glutathione (GSH) ve katalaz seviyeleri grup 3'te, grup 2'ye göre istatistiksel olarak anlamlı düzeyde yüksek bulundu (P < 0,001).

Sonuç: Bu sonuçlar CAPE'nin mekanik intestinal obstruksiyonda oluşan bakteriyel translokasyona karşı ve barsaktakta oluşan oksidatif hasara karşı koruyucu etkisinin olabileceğini göstermektedir. Bu yararlı etkinin mekanizmasını açıklamak için daha çok deneysel çalışmaya ihtiyaç vardır.

Anahtar sözcükler: Intestinal obstruksiyon, bakteriyel translokasyon, caffeic acid phenethyl ester, oksidatif stress, adrenomedullin

Introduction

Intestinal obstruction (IO) is one of the most common causes of abdominal emergencies. Strangulated obstruction, resulting in intestinal ischemia, requires emergency surgery, and early recognition is often life-saving. The mortality rates of patients with strangulated obstruction are 2-10 times higher than those of patients with nonstrangulated obstruction (1).

Bacterial translocation is described as the passage of viable bacteria from the gastrointestinal tract to extraintestinal sites without apparent rupture of the intestinal wall (2). It can be appear after IO. Although the pathogenesis of bacterial translocation is still unknown, disturbance of the homeostasis between the intestinal microflora and host defense mechanisms, such as the mucosal barrier, immunologic defense, gastric acidity, and gastrointestinal motility, can lead to bacterial translocation (3).

Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts and has been used for many years as a folk medicine. It has antiinflammatory, immunomodulatory, antiproliferative, and antioxidant properties and has been shown to inhibit lipooxygenase activities as well as suppress lipid peroxidation (4-7). Patients with suspicion of IO are generally seen in an emergency unit. The only thing done for those patients is decompression, hydration, and the stopping of oral feeding while waiting for the decision for surgery. During this time, we could give CAPE to these patients to protect them against bacterial translocation and oxidative stress. In this way, their postoperative period may have fewer complications. To date, there is no study in the literature regarding the effect of CAPE on bacterial translocation and intestinal damage after IO in rats. The aim of this study was to evaluate whether CAPE administration protects against bacterial translocation and intestinal damage in rats after IO. To assess the protective ability of CAPE in rats with IO, we measured the activities of tissue reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and adrenomedullin, and also examined bacterial translocation rates.

Materials and methods

Experimental conditions

A total of 21 male Wistar albino rats 3-months old, weighing 300-350 g, were included in this study. Experiments were done at the İnönü University Experimental Research Center. Animal experiments were performed in accordance with the National Institute of Health guidelines for animal research and were approved by the Committee of Animal Research of İnönü University, Malatya, Turkey.

Animals were housed under continuous observation in appropriate cages in a quiet, temperature-controlled (21 ± 2 °C), humidity-controlled (60 ± 5%) room, in which a 12-12 h light-dark cycle was maintained. They were allowed free access to a commercial standard diet and water ad libitum. A total of 21 rats were divided into 3 equal groups:

Group 1: Sham (n = 7)

Group 2: Intestinal obstruction (IO) (n = 7)

Group 3: IO plus CAPE (n = 7)

All surgical procedures were performed while the rats were under intraperitoneal ketamine (50 mg kg^{-1}) and xylazine HCl (10 mg kg^{-1}) anesthesia. All operations were performed under sterile conditions. Animals in Group 1 were mobilized without ligation. IO was performed by ligation of the ileum with a 4-0 silk ligature. Afterwards, the abdominal wall was closed with 2-0 silk continuous sutures.

The CAPE was synthesized by the standard method of Grunberger (8) and administered intraperitoneally once a day at a dose of 10 μmol kg^{-1} (25 μmol mL^{-1} solution in 5% ethanol). This treatment was continued for 3 days (2 days before surgery and 1 day after surgery). The animals were sacrificed in aseptic conditions 24 h after surgery. A part of the ileum was removed for biochemical analysis and stored at -85 °C until determination of MDA, GSH, CAT, and adrenomedullin. Mesenteric lymph nodes (MLN) from the terminal ileum were plated on MacConkey agar to culture gram-negative enteric bacilli, on blood agar to culture gram-positive cocci, and on selective agar to culture lactobacilli. All agar plates were incubated aerobically for 48 h at 37 °C. The culture results were determined by the number of colony-forming units per gram of tissue (CFU g^{-1}) calculated from the dilutions of organ homogenates.

Biochemical analysis

At the end of the experimental periods, rats were anesthetized by intraperitoneal injection of ketamine/xylazine hydrochloride (20/2 mg kg^{-1} body weight) and the ileum was removed immediately. The ileum tissues were separated into 2 parts. The first part was used for determination of enzyme activities and the level of GSH; the second part was used for determination of the level of lipid peroxidation. Tissue for enzyme activity studies was homogenized (PCV Kinematica Status Homogenizator) in phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Branson Sonifier 450) with 6 cycles (20 s sonications and 40 s pauses on ice). The extract was centrifuged (15,000 × *g*, 10 min, 4 °C) and cell-free supernatant was subjected to enzyme assay immediately.

For lipid peroxidation analysis, tissue was washed 3 times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were subjected to lipid peroxidation assay immediately.

Enzyme assays

The activities of CAT and glutathione peroxidase (GSH-Px) were determined spectrophotometrically. CAT activity was measured at 37 °C by following the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm (e_{240} = 40 M^{-1} cm^{-1}) (9). One unit of CAT activity was defined as the amount of enzyme catalyzing the degradation of 1 mmol min^{-1} of H_2O_2 at 37 °C and the specific activity corresponding to the transformation of substrate (in mmol min^{-1} of H_2O_2) per milligram of protein. GSH-Px activity was determined in a coupled assay with glutathione reductase by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate (10). Specific activity is given as the amount of NADPH disappeared (mmol min^{-1}) per milligram of protein.

Total glutathione (GSH) assay

The formation of 5-thio-2-nitrobenzoate (TNB) was followed spectrophotometrically at 412 nm and the amount of GSH in the extract was determined as nmol mg^{-1} of protein, utilizing a commercial GSH as the standard (11).

Lipid peroxidation assay

The analysis of lipid peroxidation was carried out as described (12) with a minor modification. The reaction mixture was prepared by adding 1 mL of homogenate to 4 mL of reaction solution (15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N NaOH; 1:1:1, w/v) and heated at 100 °C for 15 min. The mixture was cooled to room temperature and centrifuged (10,000 × *g* for 10 min), and the absorbance of the supernatant was recorded at 532 nm. MDA results were expressed as nmol mg^{-1} of protein in the homogenate.

The protein content of samples was determined using the colorimetric method of Lowry et al., using BSA as the standard (13). All analyses were performed in duplicate.

Measurement of AM level

Tissue AM concentration was measured by using high-performance liquid chromatography (HPLC). Tissue samples were subjected to reverse-phase HPLC (C-18 column, 4.6 × 250 mm, Agilent 1100), with a linear gradient dilution of CH_3CN from 10% to 60% in a solution of 0.1% trifluoroacetic acid. Human AM

was used as the standard (Sigma). The absorbance was read at 220 nm, as described previously. (14)

Statistical analysis: Data were expressed as the arithmetic mean ± SD of the number (n) of experiment; when $P < 0.05$, the difference was considered to be statistically significant. Data were analyzed statistically using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA). The results were statistically analyzed with the Kruskal-Wallis H test. The differences between groups were evaluated with Fisher's exact chi-square test.

Results

The results of bacterial translocation in groups are shown in the Table. The most common bacteria cultured from the liver and MLN of these animals were *Escherichia coli*, *Proteus mirabilis*, and *Enterococcus* spp.

The results for adrenomedullin, MDA, GSH, and CAT are shown in Figures 1-4. In the CAPE-treated rats, the MDA and adrenomedullin levels were significantly lower than in the IO group ($P < 0.001$). In the CAPE-treated rats, the ileum levels of GSH and CAT were found to be significantly higher than those in the IO group ($P < 0.001$). There was no difference between the IO group and the IO + CAPE group by pathological analysis.

Discussion

Mechanical intestinal obstruction (MIO) is one of the most common causes of acute abdominal problems and has marked effects on intestinal physiology. The impairment of normal activity of the intestinal tract by MIO may cause some important changes: bacterial overgrowth in the gut lumen, structural changes in the layers of the bowel wall, detrimental effects on blood circulation, and reduced competence of the mucosal immune function. Intestinal mucosa is a major barrier preventing the systemic spread of colonizing bacteria from the gut. Passage of viable bacteria from the gastrointestinal mucosa to the mesenteric lymph nodes and beyond has been termed bacterial translocation, which has been postulated to be a potential mechanism of systemic infection, sepsis, and multiple system organ failure. In addition to causing primary disease, bacterial translocation may negatively affect the clinical course of some patients (15,16). Many experimental and clinical studies have confirmed the translocation of bacteria during bowel obstruction (17-19).

The present study indicates that intraperitoneal administration of CAPE at a daily dose of 10 μmol kg^{-1} reduced the tissue levels of MDA and adrenomedullin but increased the levels of GSH and CAT in the ileum after intestinal obstruction. Additionally, CAPE decreased bacterial translocation in the MLN and liver after intestinal obstruction.

Table. Bacterial translocation number and rates in study groups.

Group	Bacterial translocation	P-value
Mesenteric lymph nodes (MLN)		
Sham (n = 7)	0 (0%)	0.0046[a]
Intestinal obstruction (IO) (n = 7)	6 (85%)	0.0696[b]
Intestinal obstruction (IO) + CAPE (n = 7)	4 (57%)	0.5590[c]
Liver		
Sham (n = 7)	0 (0%)	0.192[a]
Intestinal obstruction (IO) (n = 7)	3 (42%)	0.461[b]
Intestinal obstruction (IO) + CAPE (n = 7)	2 (28%)	1.000[c]

a: Sham - Intestinal obstruction
b: Sham - Intestinal obstruction + CAPE
c: Intestinal obstruction - Intestinal obstruction + CAPE

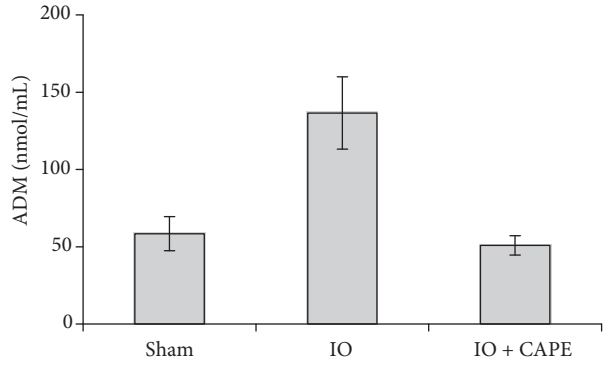

Figure 1. Tissue ADM activity was described as mean value ± SD for all groups. P < 0.001 for IO + CAPE vs. IO.
ADM: adrenomedullin, IO: intestinal obstruction, CAPE: caffeic acid phenethyl ester.
*P < 0.05 was considered to be statistically significant.

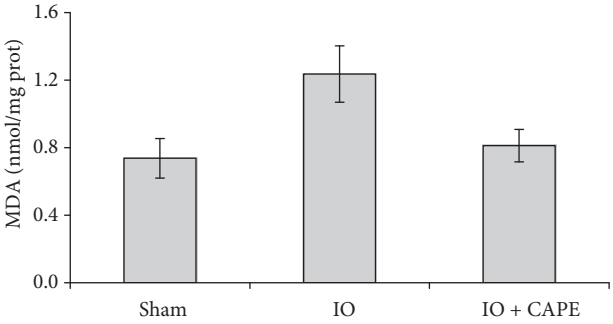

Figure 2. Tissue MDA activity was described as mean value ± SD for all groups. P < 0.001 for IO + CAPE vs. IO.
MDA: malondialdehyde, IO: intestinal obstruction, CAPE: caffeic acid phenethyl ester.
*P < 0.05 was considered to be statistically significant.

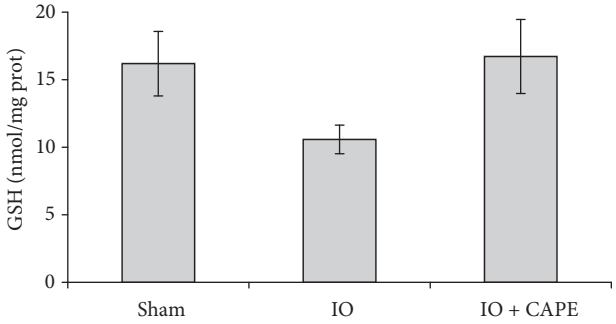

Figure 3. Tissue GSH activity was described as mean value ± SD for all groups. P < 0.001 for IO + CAPE vs. IO.
GSH: reduced glutathione, IO: intestinal obstruction, CAPE: caffeic acid phenethyl ester.
*P < 0.05 was considered to be statistically significant.

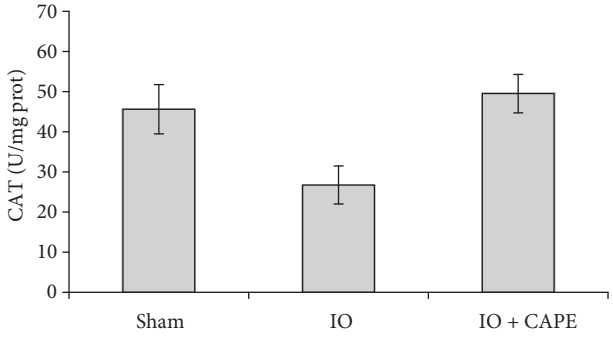

Figure 4. Tissue CAT activity was described as mean value ± SD for all groups. P < 0.001 for IO + CAPE vs. IO.
CAT: catalase, IO: intestinal obstruction, CAPE: caffeic acid phenethyl ester.
*P < 0.05 was considered to be statistically significant.

Adrenomedullin is a potent vasodilatatory peptide that was originally isolated from human pheochromocytoma (20). Ichiki et al. reported that adrenomedullin was widely distributed in human tissues, including the adrenal medulla, atrium, lung, pancreas, and small intestine (21). In addition, plasma levels of adrenomedullin were elevated under various pathologic conditions such as endotoxic shock (22), systemic inflammatory response syndrome (23), and Behçet's disease (24). In the present study, levels of adrenomedullin in the CAPE-treated rats were significantly lower than in the IO group. Different mechanisms may contribute to increased activity of adrenomedullin in rats on whom IO is performed. For instance, hypoxia (25), oxidative stress (26), and inflammatory processes may increase adrenomedullin levels (27).

MDA is a product of lipid peroxidation and is generated as a result of oxidative stress. Oxygen radicals destroy unsaturated fatty acids in the membranes (28). In the present study, levels of MDA in the CAPE-treated rats were significantly lower than in the IO group. On the other hand, the levels of GSH and CAT in the CAPE-treated rats were significantly higher

than in the IO group. The increased oxidative stress reveals the level of adrenomedullin seen in our study.

Although tissue adrenomedullin levels were clearly decreased by CAPE, the exact mechanism is not known. That CAPE directly scavenges hydroxyl radicals and thereby inhibits lipid peroxidation is well documented (5-7). Reductions in adrenomedullin levels in the CAPE-treated group is probably due to CAPE's antioxidant, free radical scavenging, antiinflammatory (28,29) effects.

In the present study, bacterial translocation in CAPE-treated rats was lower than in the IO group. The mechanism of bacterial translocation is not completely understood. The alteration of enteric flora or physical or functional injury of the intestinal barrier can all induce bacterial translocation (18). Intestinal bacterial overgrowth can also cause intestinal oxidative damage. Increased intestinal oxidative damage and bacterial translocation have been revealed in rats with IO (30). The protective effect of CAPE may be due to antiinflammatory (28) and immunomodulatory (31) activities.

A drug delivered via the intraperitoneal route can affect certain biochemical processes in the ileum wall. Studies can be done to demonstrate this effect. However, there was no difference between our study groups in the histopathological examination of their ilea.

Conclusion

The present study demonstrates that intraperitoneal administration of CAPE maintains antioxidant defenses and reduces intestinal mucosal injury, oxidative damage in the ileum, and bacterial translocation in MIO rats. This effect of CAPE may be useful to reduce bacterial translocation in patients with MIO. However, more investigations are required to evaluate CAPE's antioxidant and antiinflammatory effect in clinical and experimental models.

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